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Development of a low-cost imaging system for remote mosquito surveillance

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Abstract: Targeted vector control strategies aiming to prevent mosquito borne disease are severely limited by the logistical burden of vector surveillance, the monitoring of an area to understand mosquito species composition, abundance and spatial distribution. We describe development of an imaging system within a mosquito trap to remotely identify caught mosquitoes, including selection of the image resolution requirement, a design to meet that specification, and evaluation of the system. The necessary trap image resolution was determined to be 16 lp/mm, or 31.25um. An optics system meeting these specifications was implemented in a BG-GAT mosquito trap. Its ability to provide images suitable for accurate specimen identification was evaluated by providing entomologists with images of individual specimens, taken either with a microscope or within the trap and asking them to provide a species identification, then comparing these results. No difference in identification accuracy between the microscope and the trap images was found; however, due to limitations of human species classification from a single image, the system is only able to provide accurate genus-level mosquito classification. Further integration of this system with machine learning computer vision algorithms has the potential to provide near-real time mosquito surveillance data at the species level.

1. Introduction

Mosquito borne diseases are a global source of morbidity and mortality; in 2017 there were over 435,000 deaths due to malaria and estimated to be over 390 million dengue cases [1,2]. Dengue in particular has rapidly grown into a major global health risk. Up to 500 million people suffer from dengue each year [2], a statistic which has grown 30 fold in the last 50 years [3] . The main mosquito species responsible for transmission of dengue, *Aedes* (*Ae.*) *aegypti*, is also the primary vector for other arboviruses including Zika, yellow fever, and chikungunya, of which only yellow fever has an effective vaccine.

Essential to mosquito borne disease prevention and eventual eradication is methodical and rigorous vector control [4]. Vector surveillance, monitoring an area to understand mosquito species composition, abundance and spatial distribution, is a critically-important first step in any vector control operation because it informs decisions about what control strategies will be most effective in specific locations [5,6].

Despite its necessity, many vector control operations around the world are limited in their capacity to perform surveillance because it requires a large labor input and logistical burden. Adult mosquito surveillance operations involve placing numerous mosquito traps in a region, returning to each trap the next day to collect specimens, and driving them to a lab. There, specimens are identified individually under a microscope by trained staff. This laborious process limits the quantity of data, producing inadequate spatial distribution of data. It has been shown

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through retrospective analysis that if vector surveillance data could be gathered in high density and real time, dengue incidence can be predicted ahead of time to inform control activities [7].

Mosquito traps use a variety of methods to attract and capture mosquitoes (Fig. 1.). Two reliable methods for capturing adult container-breeding *Aedes* mosquitoes, including *Ae. aegypti* and *Ae. albopictus* are the BG-Sentinel (BGS) and the Gravid *Aedes* Trap (GAT) [8–10]. The GAT attracts mosquitoes through breeding site visual and chemical cues (water in a small dark container), and captures and kills mosquitoes through either lethal pyrethroid contact, manipulating light cues to prevent mosquitoes from escaping, or a sticky paper inside the trap [11]. The BGS attracts mosquitoes with visual cues, CO2, and chemical attractants, and captures mosquitoes using a fan and net [8]. A key distinction between the two traps is that the BGS is an active trap, requiring a power source to run, while the GAT is a passive trap, not requiring a power source. Additionally, the GAT catches fewer mosquitoes than the BGS. Though both of these traps are reliable at attracting container breeding *Aedes* mosquitoes, both also attract other species of mosquitoes [8,9]. The varied vectoral capacities and behaviors of different species caught necessitates identification of individual specimens under a microscope.

One instance where this is particularly true is distinguishing between *Aedes* species. *Ae. aegypti* commonly competes with *Ae. albopictus* in urban environment breeding sites [12]. These

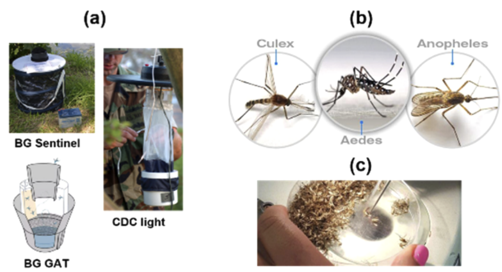

Fig. 1. The current workflow of vector surveillance involves (a) Mosquito specimens are caught using a variety of methods; (b) Entomologists morphologically identify mosquito specimens by species; and (c) Hundreds to thousands of mosquitoes are individually sorted under a microscope.

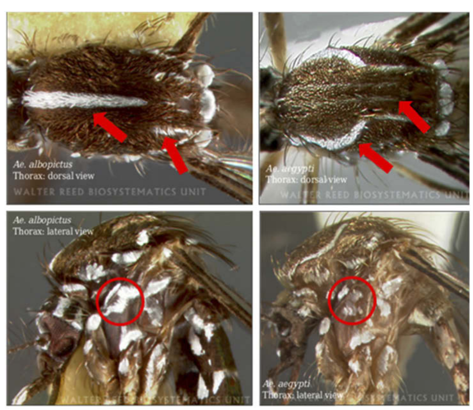

Fig. 2. *Aedes aegypti* and *Aedes albopictus* can be differentiated by examining the stripes on the dorsal view of the thorax (red arrows), and the spots on the lateral view of the thorax (red circle) [14]. Trained technicians and entomologists examine features like these to determine a mosquito's species. Determining species informs mosquito control operations. (© 2019 Walter Reed Biosystematics Unit, Smithsonian Institute)

two species are morphologically similar but can be differentiated through visual examination of the thorax (Fig. 2). *Ae. albopictus* is capable of transmitting arboviruses; however, is considered a less capable vector than *Ae.* a*egypti* [13]. Therefore, determining the abundance difference between these two species in an area is critical to determining disease transmission risk or outbreak potential.

In this report, we describe a low-cost imaging system that captures high resolution images of mosquitoes immobilized in a mosquito trap. A GAT which uses a sticky paper as the specimen capture mechanism was selected for this system to provide a uniform object surface for imaging and a low capture rate to minimize overlapping mosquitoes for imaging. Such a system might significantly improve the logistical efficiency of mosquito surveillance, no longer requiring a surveillance team to physically visit a trap for every trap catch data point. We describe the necessary design specifications, a subsequent optical design, and initial evaluation of the optical performance for remote surveillance.

2. Methods

2.1. Determination of resolution specifications

Ae. aegypti can be distinguished from *Ae. albopictus* by the pattern of white scales on the dorsal and lateral view of the thorax (Fig. 2) [14]. The smallest of these features are the separated white spots on the lateral view of the thorax of the *Ae. aegypti*, contrasting with the connected white spots visible on the lateral view of the thorax of *Ae. albopictus*. Using microscopic photos, four desiccated lab-reared *Ae. aegypti* specimens were measured in ImageJ [15] and pixel approximations were used to estimate the minimum resolution necessary to visualize the dots on the lateral view of the thorax. Although some genetic variation may exist within species, and between lab bred and field caught mosquitoes, measuring the distance between lateral dots provides a conservative estimate of minimum necessary resolution because there are many other distinguishing features between the two species with larger measurable differences. The minimum resolution necessary for classification for each specimen was determined by examining the smallest feature of each specimen. Feature size was determined by placing a metric ruler in the image and determining pixel size using measurement tools in ImageJ. The mean and standard deviation minimum resolution was calculated to determine a ranged requirement. Morphological features of *Cu. quinquefasciatus* and *An. funestus* were not used to determine the minimum resolution because they are very morphologically different from each other and from the *Aedes* species used; therefore, any large differences would not be relevant.

In order to cross validate the resolution determined above, a survey was distributed to entomologists to determine the necessary resolution for identification across a variety of mosquito species using an image. Entomologists were presented with 32 unique specimen images at different resolutions to determine the necessary resolution threshold for humans to identify mosquito species from an image. Entomologists were asked to give a response, regardless of how confident they were of their answer. The specimens provided in the survey were imaged at high resolution with a handheld microscope (Zarbeco, Miscope), measured resolution of 28.51 lp/mm (line pairs/mm) and theoretical resolution of 32 lp/mm, then down sampled by half with Gaussian smoothing 4 times. Images from this set were chosen at random to be classified by entomologists. The survey spanned 12 mosquito species from 5 mosquito genera. Statistical significance was examined on genus and species accuracy independently using the chi-square test between consecutive resolution groups.

2.2. Optical system design

GATs often capture and kill mosquitoes using a sticky paper mounted on the wall of the GAT, which is conical at a 5° angle from the vertical except for the bottommost 30 millimeters, which are cylindrical in shape. In order to effectively capture an image of the sticky paper without obstruction from other trap components, the sticky paper must be aligned with the bottom of the trap. When placed on the wall aligned with the bottom of the trap, a portion of the sticky paper is cylindrical in surface shape as described above (Fig. 3). This geometry necessitates a large depth of field to capture an image of the sticky paper surface at a consistent resolution through the entire field of view.

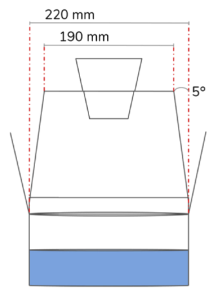

Fig. 3. The internal wall of the Gravid *Aedes* Trap (GAT) is partially cylindrical on the bottom-most part of the inside wall, and majority conical at 5°. A sticky paper is the mosquito capture mechanism for the GAT and is placed on this complex surface. This provides immobilization of mosquito specimens and a surface to image.

The resolution at points across the sticky paper was verified using a 1951 USAF Resolution Test Target, placed at various optically unique locations on the sticky paper. The minimum and maximum resolution locations were identified via visual analysis, judging resolution as the highest discernible element, and group number representing a specific line pairs per millimeter (lp/mm). The percent contrast at each group element in the resolution test target was determined by plotting the profile of the three line pairs of each group element. The pixel values of the center two peaks and the center trough were measured and evaluated with the following function:

$$\text{Contrast} = 1 - (2 \times t_c/(p_1 - p_2)) \tag{1}$$

Where t_c is the center trough average pixel value, p_1 is the first peak average pixel value, and p_2 is the second peak average pixel value. The average contrast of the three photos for each resolution group element was reported.

2.3. Mosquito test samples

Lab bred mosquitoes from species *Aedes aegypti* [Rockefeller strain], *Anopheles gambiae sensu stricto (s.s.)* [Keele strain], and *Culex quinquefasciatus* [Johannesburg strain and a Hainan strain] were all captured on sticky papers placed inside their colony boxes, and then imaged with the microscope (Zarbeco miscope) and trap imaging system within 24 hours of capture.

2.4. Evaluation protocol

Trap image quality was verified by comparing the identification accuracy of entomologists given specimen images from a microscope versus the optical system of the trap. Specimens were imaged in the trap in ideal lighting. Trap images were cropped to include only the specimen of interest. Trap images and microscope images were paired and placed in a survey in random order to be evaluated by entomologists with expertise in mosquito identification. Entomologists were shown the trap image first and asked to choose the correct species from a list of nine species, then shown the microscope image and asked to choose the correct species from the same list. The list of possible species included two commonly confounding species for each species present: *Ae.*

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aegypti, Ae. albopictus, Ae. japonicus, Cu. erraticus, Cu. pipiens, Cu. quinquefasciatus, An. funestus, An. gambiae, and *An. stephensi*. Entomologists were required to give an answer for each image, regardless of their confidence in the identification. The ability to identify species from trap images compared to microscope images was evaluated using McNemar's test. Both genus and species discordance was tested if the total discordant pairs were higher than 30. When the discordant samples were fewer than 30, the proportion of correctly identified samples from the trap and microscope were compared by calculating the z score for the difference between two proportions [16,17].

3. Results

3.1. Design specification

The ventral view of four desiccated colony specimens of *Ae. aegypti* were imaged and the distinguishing thoracic spot features were measured to have an average minimum feature size of 37.8 ± 4.0 µm (Fig. 4). The calculated minimum resolution (mean and standard deviation) for capturing these features was 13.34 ± 1.35 lp/mm. Assuming a normal distribution of the size of features among specimens, z-scores of 1, 1.5, and 2 indicate that 84.1%, 93.3%, and 97.7% of samples will have visible features with a resolution of 14.69, 15.37, and 16.04 lp/mm.

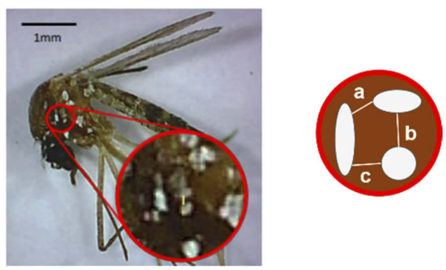

Fig. 4. The first method of determining the resolution requirement: direct measurement of features of interest on the specimen. The minimum distance between the three spots of primary interest on the *Ae. aegypti* specimen was determined (a, b, or c, depending on the specimen). The mean and standard deviation of four specimen measurements was used to determine a minimum feature size of 37.8 +- 4.0 µm.

When assessing entomologist's ability to correctly identify mosquitoes at varying resolutions, a chi-square test between consecutive resolutions confirmed significant differences between 16 and 8 lp/mm in both the genus ($p = 0.0004$) and species ($p=0.00004$) groups. There were no other significant differences between consecutive resolution groups (Table 1).

Table 1. Entomologist identification capabilities at depreciating resolutions.

Calculated lp/mm	Measured lp/mm	Samples[a]	Genus Accuracy	Species Accuracy
32	28.51	36	83.33%	52.78%
16	16.00	44	88.64%	61.36%
8	7.13	44	54.55%	18.18%
4	3.17	30	50.00%	30.00%
2	1.41	11	54.55%	9.09%

[a]Entomologists were shown 32 images of different mosquitoes at depreciated resolutions consisting from the following species: *Aedes (Ae.) albopictus, Ae. sollicitans, Ae. taeniorhynchus, Anopheles (An.) crucians, An. punctipennis, An. quadrimaculatus, Coquillettidia (Co.) perturbans, Culex (Cu.) erraticus, Cu. coronator, Psorophora (Ps.) columbiae, Ps. ferox, Ps. pygmaea*.

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Using these two methods, it was determined that the trap image system must be able to capture a 16 lp/mm, or 31.25um image.

3.2. Optics design

Due to the complex geometry of the trap wall, a large depth of field was desired to minimize differences in resolution throughout the sticky paper. Depth of field was maximized by placing the imaging system as far away from the sticky paper as possible and selecting a high f-stop number. The image plane was made to be parallel to the majority of the inner wall of the trap. To cover the 80mm x 120mm sticky paper for a minimum cost, two ¼" frame sensors were used with a pixel dimension of 3264×2448. These sensors, together with 10mm, F/4 lenses, capture a field of view (FOV) of 72mm x 104mm when placed in the described location (Fig. 5). This FOV is 77% smaller by area than the size of the sticky paper provided with the BG-GAT, but the size of the sticky paper can be modified with little expected impact to the capture rate of the trap.

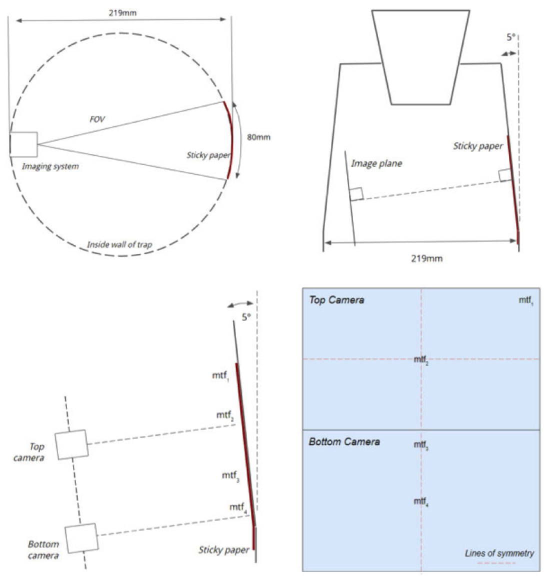

Fig. 5. Camera placement given optical constraints. The curved geometry of the wall of the GAT places mosquitoes caught on the sticky paper at different distances from a camera in the trap, requiring the camera to have a large depth of field to include the full sticky paper at a similar resolution. The authors achieved this by placing the camera as far from the sticky paper within the trap as possible, as well as through lens selection. The bottom right image shows the locations within the field of view measured in subsequent testing.

The minimum and maximum resolution of the top and bottom cameras was evaluated by measuring resolution at four points shown above (MTF1, MTF2, MTF3, MTF4), resulting in the modulation transfer functions shown in Fig. 6. The minimum resolution throughout the FOV was captured at MTF1, the bottom right corner of the top camera, with a 10% contrast resolution determined to be 14.73 lp/mm through linear interpolation of the modulation transfer function. The maximum resolution was captured at MTF2, the center position of the bottom camera, with a 10% contrast resolution determined to be 20.06 lp/mm through linear interpolation. The minimum and maximum of the bottom camera, MTF3 and MTF4, had a 10% contrast determined to be 15.71 and 18.95 lp/mm.

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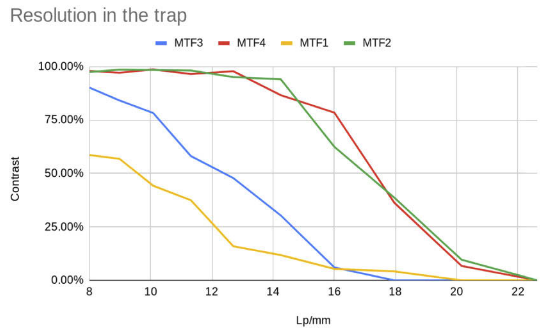

Fig. 6. Modulation Transfer Function of the minimum and maximum resolution location of the top and bottom cameras. The minimum resolution in the field of view with a contrast of 10% was 14.73 lp/mm. The average throughout the field of view was 17.36 lp/mm, reaching the resolution requirement determined above in the majority of the field of view.

3.3. Resolution evaluation

Three entomologists completed the survey of 50 non-overlapping paired samples from each species, resulting in 446 samples between the three species (Fig. 7). Four samples were excluded from analysis because the entomologist did not provide an answer for one of the image types. Using McNemar's test, uniform discordance was measured between the trap and microscope images for species classification (Table 2). McNemar's test could not be used for

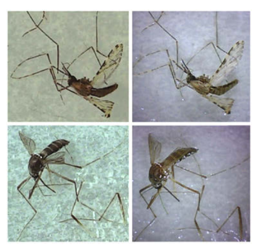

Fig. 7. Examples of paired images shown in the Resolution Verification Survey, where entomologists were shown first an image from our system in the trap and asked to identify the species, and then a microscope image of the same specimen and asked to identify the species.

Table 2. Resolution validation survey results genus and species classification abilities given microscope images and our trap system images. Three entomologists were tested on three species.

	Total		*Anopheles gambiae s.l.*		*Aedes aegypti*		*Culex quinquefasciatus*	
				Species				
Sample size	446		150		150		146	
Trap images correctly identified	209	46.86%	89	59.33%	101	67.33%	17	11.64%
Microscope images correctly identified	209	46.86%	88	58.67%	104	69.33%	15	10.27%
				Genus				
Sample size	446		150		150		146	
Trap images correctly identified	437	97.98%	148	98.67%	149	99.33%	140	95.89%
Microscope images correctly identified	443	99.33%	147	98.00%	150	100.00%	146	100.00%

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genus classification accuracy because there were only 11 discordant samples. In this case, there was a statistical difference in overall genus classification accuracy by entomologists between the microscope images and the trap images: 99.33%, 97.98%, p=0.0409 (Table 3); however, accuracy for both images types was very high.

Table 3. Discordance of entomologist mosquito classification abilities between trap images and microscope images.[a]

Species		Microscope	
		correct	incorrect
Trap	correct	183	26
	incorrect	26	215
Genus		Microscope	
		correct	incorrect
Trap	correct	435	3
	incorrect	8	0

[a]The discordance measured between microscope and trap image classification was measured to be uniformly distributed between samples for species classification, showing the lack of difference between image types for species classification.

4. Discussion and Conclusion

A resolution design specification of 16 lp/mm, or 31.25um was determined by measuring significant features on desiccated *Ae. aegypti* specimens, validated through an entomologist survey measuring classification accuracy over varying resolutions. An imaging system was designed which, across the desired FOV of the sticky paper, achieves 14.73 lp/mm at minimum, and averages 17.36 lp/mm at 10% contrast. Although the minimum resolution for each camera was below the resolution design specification of 16 lp/mm at 10% contrast, this had no measured impact on species classification results by entomologists. Uniform discordance was measured for entomologist species classification capabilities based on trap and microscope images. In the genus classification, there was a statistically significant difference measured between classification abilities with trap images compared to microscope images; however, both microscope and trap classification were extremely high in accuracy, 99.33% and 97.98%.

These results provide preliminary validation for the use of such a system in remote monitoring of mosquito population density and diversity. Regardless of limitations in species identification accuracy, information on the genus of the specimens captured in the trap may prove valuable. There is still a significant reported need for continuous local monitoring of populations of *Ae. albopictus* and *Ae. aegypti* [18], and reducing the frequency of trap site visits would significantly reduce the logistical burden of vector surveillance. Remote monitoring could enable faster mosquito data acquisition, further enabling disease prediction models and faster vector control responses. Further work on the value of genus level population diversity and density data is required to validate the effectiveness of this system.

The primary limitation of this work is the low species classification accuracy achieved by the human entomologists on both the microscope and the trap images. Visual identification of mosquito species is the most common method used to identify specimens in operational mosquito surveillance. This is normally performed with the specimen under a microscope, allowing an entomologist to manipulate the mosquito to view different orientations of the mosquito and see all relevant features to visual taxonomic identification. However, visual identification is reported in some instances to be a difficult and unreliable task [19,20]. Although the low accuracy of species identification by entomologists measured in this work is similar to that observed in other studies, it may be impacted by the use of images rather than in-person examination of

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the specimen. Additionally, entomologists in this study were only given one orientation from which to identify the specimen species. These two factors may have impacted the accuracy of entomologist identification. Further evaluation is necessary to determine the impact of this limitation, and validate the effectiveness of this remote monitoring method.

Although the human classification accuracy measured in this study had low accuracy, some groups have shown the promise of recent advances in computer vision to provide a solution to the difficult problem of mosquito species identification [21,22]. These studies were limited to a small species set, which is not representative of the classification task in operational surveillance; however, these results warrant further evaluation of such computer vision methods on a larger, more representative species set. If successful, a computer vision method could be valuable paired with this remote monitoring system. Whether relying on computer vision or entomologist examination, a remote imaging system would need to include internet of things electronics to transmit images to a centralized location. To provide the most value, these electronics would need to be optimized for long duration field deployments, using power saving electronics and a ruggedized design for extreme weather.

In this work we have demonstrated the feasibility of an optical system used for remote identification of mosquitoes captured in a trap. The logistical burden of servicing numerous traps in a region has severely limited the development of robust mosquito surveillance systems around the world. Even in the United States, where there are significant preventative public health budgets available, only 61% of mosquito surveillance districts enact their control treatments based on mosquito surveillance data [23]. In the Western Pacific region, where dengue is a significant concern, many countries do not engage in entomological surveillance on a routine basis [24]. A system similar to that presented in this work could reduce the logistical burden required to engage in vector surveillance at a meaningful scale to inform vector control activities in an area, and enable the expansion of vector surveillance activities.

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Disclosures

The Authors disclose that the following authors have conflict of interest due to affiliation with VecTech LLC, which has an interest in technologies aimed at empowering the fight against mosquito borne disease: Tristan Ford, owner and officer; Margaret Glancey, officer; Adam Goodwin, officer.

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